Genetic diversity and relationships in accessions from different cultivar groups and origins in the tree tomato (*Solanum betaceum* Cav.)

Pablo G. Acosta-Quezada · Santiago Vilanova · Juan B. Martínez-Laborde · Jaime Prohens

Abstract Tree tomato (*Solanum betaceum*) is an Andean small tree cultivated for its juicy fruits. Little information is available on the characterization of genetic resources and breeding of this neglected crop. We have studied the molecular diversity with AFLP markers using 11 combinations of primers of a collection of 25 *S. betaceum* accessions belonging to four cultivar groups, most of which had been previously morphologically characterized, as well as one accession of the wild relative *S. cajanumense*. A total of 197 AFLP fragments were scored, of which 84 (43 %) were polymorphic. When excluding *S. cajanumense* from the analysis, the number of polymorphic AFLP fragments was 78 (40 %). Unique AFLP fingerprints were obtained for every accession, but no AFLP fragments specific and universal to any of the four cultivar groups were found. The total genetic diversity (H_T) of cultivated accessions was $H_T = 0.2904$, while for cultivar groups it ranged from $H_T = 0.1846$ in the orange group to $H_T = 0.2498$ in the orange pointed group. Genetic differentiation among cultivar groups (G_{ST}) was low ($G_{ST} = 0.2248$), which was matched by low values of genetic distance among cultivar groups. The diversity of collections from Ecuador, which we hypothesize is a center of diversity for tree tomato, was similar to that from other origins ($H_T = 0.2884$ and $H_T = 0.2645$, respectively). Cluster and PCoA analyses clearly separated wild *S. cajanumense* from the cultivated species. However, materials of different cultivar groups and origins were intermingled in both analyses. The Mantel test correlation coefficient of the matrices of morphological and AFLP distances was low (−0.024) and non-significant. Overall, the results show that a wide diversity is present in each of the cultivar groups, indicate that Ecuador may be regarded as a center of accumulation of diversity for this crop, and confirm that AFLP and morphological characterization data are complementary. The results obtained are of value for the conservation of genetic resources and breeding of tree tomato, as an assessment of the genetic diversity and relationships among different cultivar groups and geographic origins is obtained.

Keywords AFLPs · Conservation · Cultivar groups · Genetic resources · *Solanum betaceum* · Tree tomato

Introduction

The tree tomato (*Solanum betaceum* Cav.), also known as tamarillo, is a small tree cultivated for its edible, slightly acid, fruits (Bohs 1989; Prohens and Nuez 2000). Tree tomato fruits have a high content of ascorbic acid, provitamin A carotenoids, and vitamin B_6, as well as a high antioxidant activity (Vasco et al. 2009), and are mostly consumed in juices after being peeled and squeezed with water or milk. Although tree tomato cultivation and consumption is popular in many places in its native home in the Andean region of South America (Bohs 1989), it is considered as a neglected crop (National Research Council 1989). In New Zealand, the tree tomato crop was introduced as a new exotic fruit crop and its production and export have increased during the last decades (Boyes and Strübi 1997; Prohens and Nuez 2000). In citrus growing areas of the Mediterranean regions of countries like Spain or Italy it has been envisaged as a promising alternative fruit crop (Prohens et al. 2004). In US markets it is a minor fruit oddity.

Despite this interest and potential, research on tree tomato diversity, conservation, and breeding has been limited (Bohs 1989; Pringle and Murray 1991; Cohen et al. 2000; Enciso-Rodríguez et al. 2010; Acosta-Quezada et al. 2011). To our knowledge, there are only two previous reports of the study of the morphological (Acosta-Quezada et al. 2011) and molecular (Enciso-Rodríguez et al. 2010) diversity of a meaningful (i.e., >20) number of accessions. In the morphological study, we developed and used 39 quantitative descriptors to assess the diversity of 24 tree tomato accessions from five cultivar groups which originated in different countries (Acosta-Quezada et al. 2011). Among these accessions we identified some agronomically interesting accessions, i.e., with good fruit set, large fruits, and attractive fruit shape, and found a considerable diversity among accessions for most of the descriptors, with the greatest variation and heritability values occurring for fruit traits. Furthermore, we found a low morphological differentiation among cultivar groups. Enciso-Rodríguez et al. (2010) studied a collection of 26 accessions (mostly of Colombian origin) of *S. betaceum* with five polymorphic orthologous nuclear markers. Using this limited number of markers, these authors found a high degree of homozygosity and a high genetic differentiation (almost no sharing of alleles) among accessions. Unfortunately, no information was provided about the cultivar group of the accessions used for this molecular characterization.

The availability of morphological and molecular characterization of the genetic resources diversity is of great relevance for the enhancement and breeding of tree tomato, as both types of characterization provide distinct and complementary information (Hillis 1987; Mohammadi and Prasanna 2003). The present study examines AFLP variation of the accessions used in the morphological study of Acosta-Quezada et al. 2011. As little genomic information is currently available for tree tomato, and no SSR or SNP markers have been developed for this crop, we used AFLP markers, which do not require previous genomic information (Meudt and Clarke 2007). AFLPs have the advantage that in each run a high number of fragments can be scored and that they have a higher reproducibility than some other markers, like RAPDs, and do not require genomic information (Powell et al. 1996; Jones et al. 1997). AFLPs have been successfully used for assessing the genetic diversity of other *Solanum* crops from the Andean region, like potato (*Solanum tuberosum* L.), tomato (*Solanum lycopersicum* L.), or pepino (*Solanum muricatum* Aiton) (Milbourne et al. 1997; Tam et al. 2005; Blanca et al. 2007). They have also been used to establish breeding and conservation strategies for horticultural crop landraces (Portis et al. 2012). The information obtained will be relevant for the conservation of genetic resources and to helping identify breeding strategies and opportunities for the tree tomato.

Materials and methods

Plant material

A total of 26 tree tomato accessions, of which 25 correspond to cultivated *S. betaceum*, and one to the wild relative *S. cajanumense* Kunth (outgroup), were used for AFLP characterization (Table 1). The cultivated accessions belong to four cultivar groups as defined by Acosta-Quezada et al. (2011): orange, orange pointed, purple, and red. Twenty two of the *S. betaceum* accessions used here were previously morphologically characterized (Acosta-Quezada et al. 2011). The plant material used in this study was originally collected in seven countries and provided by

Table 1 Tree tomato accessions studied, cultivar group, origin (including province or department and country), and availability of morphological characterization data (Acosta-Quezada et al. 2011)

Accession	Germplasm bank	Accession code in germplasm bank	Cultivar group	Origin	Morphological characterization
A15	UTPL	ECUts-100	Wild	Loja (Ecuador)	
A17	UPV	ECU-1221	Orange pointed	Azuay (Ecuador)	X
A18	UPV	ECU-1248	Red	Tungurahua (Ecuador)	X
A19	UPV	ECU-1295	Orange pointed	Carchi (Ecuador)	X
A20	UPV	ECU-1567	Orange pointed	El Oro (Ecuador)	X
A21	UTPL	ECUt-001	Purple	Loja (Ecuador)	X
A22	UTPL	ECUt-002	Orange	Azuay (Ecuador)	X
A23	UTPL	ECUt-003	Orange pointed	Azuay (Ecuador)	X
A24	UTPL	ECUt-004	Red	Azuay (Ecuador)	X
A25	UTPL	ECUt-005	Purple	Azuay (Ecuador)	X
A26	UTPL	ECUt-006	Red	Tungurahua (Ecuador)	X
A27	UTPL	ECUt-007	Red	Tungurahua (Ecuador)	X
A28	UPV	SUD-CY-1	Red	Manizales (Colombia)	
A29	UTPL	ECUt-008	Orange	Cotopaxi (Ecuador)	X
A30	UPV	QB-54	Purple	Boyacá (Colombia)	X
A31	UPV	UNT-08	Orange pointed	Lima (Peru)	X
A32	UPV	PT-087	Orange pointed	Chachapoyas (Peru)	X
A33	UPV	PT-221	Orange pointed	Cajamarca (Peru)	X
A34	UPV	PT242	Orange pointed	Cajamarca (Peru)	X
A35	UPV	BOL-14	Orange pointed	Cochabamba (Bolivia)	X
A36	UPV	BOL-116	Orange pointed	Santa Cruz (Bolivia)	X
A37	UPV	EUR-CY-1	Purple	Lisboa (Portugal)	X
A38	UPV	UPV023113	Orange	Valencia (Spain)	
A39	UPV	NZ-1	Purple	New Zealand	X
A40	UPV	NZ-2	Purple	New Zealand	X

Table 2 Selective primers, restriction enzyme, sequence and fluorescent label for the oligonucleotide pre-selective and selective primers used in AFLP characterization

Primers		Restriction enzyme	Sequence	Fluorescent label
*Eco*RI	*Mse*I			
ACT	CAA	*Eco*RI	5′-GACTGCGTACCAATTCACT-3′	Fam
		*Mse*I	5′-TGAGTCCTGAGTAACAA-3′	
ACT	CCA	*Eco*RI	5′-GACTGCGTACCAATTCACT-3′	Fam
		*Mse*I	5′-TGAGTCCTGAGTAACCA-3′	
AGC	CAA	*Eco*RI	5′-GACTGCGTACCAATTCAGC-3′	Hex
		*Mse*I	5′-TGAGTCCTGAGTAACAA-3′	
ACG	CAA	*Eco*RI	5′-GACTGCGTACCAATTCACG-3′	Ned
		*Mse*I	5′-TGAGTCCTGAGTAACAA-3′	
ACC	CAC	*Eco*RI	5′-GACTGCGTACCAATTCACC-3′	Fam
		*Mse*I	5′-TGAGTCCTGAGTAACAA-3′	
AGG	CAC	*Eco*RI	5′-GACTGCGTACCAATTCAGG-3′	Hex
		*Mse*I	5′-TGAGTCCTGAGTAACAC-3′	
AAC	CAC	*Eco*RI	5′-GACTGCGTACCAATTCAAC-3′	Ned
		*Mse*I	5′-TGAGTCCTGAGTAACAC-3′	
ACA	CAC	*Eco*RI	5′-GACTGCGTACCAATTCACA-3′	Fam
		*Mse*I	5′-TGAGTCCTGAGTAACAC-3′	
AAG	CTC	*Eco*RI	5′-GACTGCGTACCAATTCAAG-3′	Ned
		*Mse*I	5′-TGAGTCCTGAGTAACTC-3′	
AGC	CTC	*Eco*RI	5′-GACTGCGTACCAATTCAGC-3′	Hex
		*Mse*I	5′-GAGTCCTGAGTAACTC-3′	
ACG	CCG	*Eco*RI	5′-GACTGCGTACCAATTCACG-3′	Ned
		*Mse*I	5′-TGAGTCCTGAGTAACCG-3′	

Table 3 Number of polymorphic AFLP markers from 25 cultivated tree tomato accessions (*S. betaceum)* and one wild relative (*S. cajanumense*) using eleven primer combinations and two restriction enzymes

Primers		Markers (*n*)	Polymorphic markers (*n*)	Polymorphic markers (%)	Range (bp)
*Eco*RI	*Mse*I				
ACT	CAA	14	5	35.7	92–207
ACT	CCA	20	14	70.0	66–303
AGC	CAA	18	6	33.3	56–224
ACG	CAA	11	2	18.2	71–129
ACC	CAC	23	10	43.5	70–206
ACA	CAC	26	19	73.1	71–260
AGG	CAC	17	5	29.4	100–145
AAC	CAC	12	4	33.3	70–117
AAG	CTC	15	3	20.0	70–150
AGC	CTC	28	11	39.3	92–205
ACG	CCG	13	5	38.5	80–209
Total		197	84	42.6	56–303
Average		17.9	7.6		

the germplasm banks of the Universidad Técnica Particular de Loja (UTPL) in Ecuador and of the Universidad Politécnica de Valencia (UPV) in Spain; the material was selected to represent a diversity of cultivar groups and geographical origins, and an special emphasis was made in including Ecuadorian

accessions, as a wide variation has been reported in this country (Bohs 1989). An ample representation of Ecuadorian accessions was included in order to study the diversity present in this country compared to that of other origins, comprising Peru, Colombia, Bolivia, New Zealand, Portugal, and Spain (Table 1).

Molecular characterization

For each accession, genomic DNA was isolated from a mixture of young leaves from five plants evaluated using the CTAB method (Cetyl Trimethyl Ammonium Bromide) (Doyle and Doyle 1987). DNA concentration was quantified on agarose, and a 0.1 μg DNA sample was digested by the enzyme combination *Eco*RI and *Mse*I at 37 °C for 2.5 h. Ligation was performed with the AFLP Core Reagent Kit (Invitrogen Corp., Carlsbad, California, USA) following the instructions of the manufacturer. After ligation, the reaction mixture was diluted 1:10 in Tris–EDTA (TE) buffer.

For the preselective amplification, a 5 μl aliquot from the 1:10 DNA dilution was added to a 25 μl solution containing 2.5 μl of 10× buffer, 1 μl of MgCl (25 mM), 0.5 μl of primer *Eco*A (10 mM), 0.5 μl of primer *Mse*C (10 mM), 1.0 μl of dNTPs (10 mML), and 0.8 U of *Taq* polymerase (Roche, Basel, Switzerland). After preamplification, DNA was diluted another time 1:10 in TE buffer. The selective amplification was performed on 2 μl aliquots of the former solution using eleven combinations of primers (Table 2). DNA fragments were separated in an ABI/PRISM® 377 genetic analyzer (Applied Biosystems, Foster City, California, USA). Resulting fragments were scored as binary traits (1 = present, 0 = absent) using Genographer v. 2.1.4. (Benham et al. 1999).

Data analysis

Pairwise genetic similarities were estimated with the Dice similarity coefficient $S_{ij} = 2a/(2a + b + c)$, where a is the number of bands shared by i and j, b is the number of bands present in i and absent in j, and c is the number of bands present in j and absent in i (Mohammadi and Prasanna 2003). The resulting genetic similarity matrices were used to generate an unweighted pair group method using arithmetic means (UPGMA) phenogram (Sneath and Sokal 1973); goodness of fit of the resulting phenogram was evaluated with the cophenetic correlation coefficient by using the Mantel (1967) test. In addition, a principal coordinate analysis (PCoA) was performed. UPGMA and PCoA analyses were conducted using the NTSYSpc2.0 (Applied Biostatistics Inc., Setauket, NY, USA) software package.

Genetic diversity in the *S. betaceum* cultivar groups (orange, orange pointed, red, and purple) and in different geographic origins (Ecuador vs. other origins) was estimated with the total genetic diversity (H_T) (Nei 1973). Total diversity was partitioned into diversity among (D_{ST}) and within (H_S) groups. The relative magnitude of genetic differentiation among groups (G_{ST}) was calculated as the ratio D_{ST}/H_T (Nei 1973). Genetic distances among the *S. betaceum* cultivar groups were calculated according to Nei (1972). Calculations of genetic diversity and genetic distances for the cultivar groups were performed using the POPGENE 3.2 software (Yeh and Boyle 1997).

In order to study the relationship between morphological (using data obtained by Acosta-Quezada et al. 2011) and molecular (using AFLP data from this work) differences among accessions, a Mantel (1967) test was performed by comparing the distance

Table 4 Genetic diversity statistics of 25 accessions of cultivated tree tomato (*S. betaceum*) estimated from AFLP data, grouped according to cultivar groups (orange, orange pointed, orange, purple and red) and to geographical origin (Ecuador vs. other origins)

Groups	Accessions (*n*)	H_T	D_{ST}	H_S	G_{ST}
Cultivar groups					
Total	25	0.2904	0.0653	0.2251	0.2248
Orange	3	0.1846			
Orange pointed	11	0.2498			
Purple	6	0.2413			
Red	5	0.2246			
Origins					
Total	25	0.2904	0.0140	0.2764	0.0712
Ecuador	13	0.2884			
Other origins	12	0.2645			

H_T total genetic diversity, D_{ST} among groups genetic diversity, H_S within groups genetic diversity, G_{ST} relative magnitude of genetic differentiation among groups

Table 5 Genetic distance among cultivated tree tomato (*S. betaceum*) cultivar groups (orange, orange pointed, purple, and red)

Cultivar groups	Orange	Orange pointed	Purple
Orange pointed	0.1106		
Purple	0.1667	0.0986	
Red	0.1665	0.0840	0.0869

(Euclidean) matrix based on morphological data and the genetic distance (Dice) matrix for the 22 accessions of *S. betaceum* used in both studies. Calculations were performed using the NTSYSpc2.0 software.

Results

AFLP analysis

The 11 AFLP primer combinations yielded a total of 197 fragments ranging from 56 to 303 bp, of which 84 (43 %) were polymorphic and informative (Table 3). For cultivated *S. betaceum*, i.e., excluding the wild *S. cajanumense* accession A15, the number of polymorphic loci was 78 (40 %). The number of polymorphic AFLP markers obtained by each combination of primers ranged between 2 and 19 (Table 3). The combinations with a greater number of polymorphic loci were ACA/CAC (19 markers) and ACT/CCA (14 markers).

The polymorphic AFLP fragments have allowed obtaining a unique genetic fingerprint for every accession. When studying the number of AFLP differences among pairs of accessions, we have found that the differences between the wild accession A15 and the cultivated accessions ranged between 51 (with accessions A25 and A33) and 24 (with accession A40). When only the cultivated accessions were taken into account, the number of differences among accessions has ranged between 41 (for accessions A26 and A38, the former belonging to the red group and the latter to the orange group) and 7 (for accessions A39 and A40, both of which belong to the purple group). The wild accession A15 presented one AFLP fragment which was absent from cultivated material and lacked five AFLP fragments that were universal to *S. betaceum* material. However, no AFLP fragments specific and universal to cultivar groups (i.e., present in all accessions of one group and absent from all other groups) were found.

Genetic diversity

The total genetic diversity (H_T) of the 26 accessions was $H_T = 0.3194$. When excluding the wild accession A15, the genetic diversity of the 25 cultivated accessions was $H_T = 0.2904$. When considering the

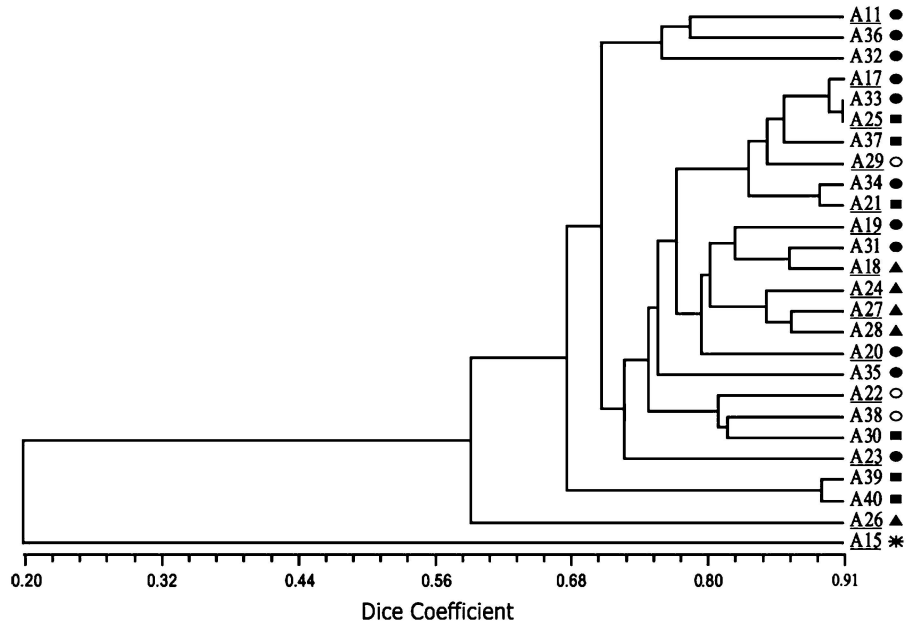

Fig. 1 UPGMA phenogram of 25 cultivated tree tomato accessions (*S. betaceum)* and one wild relative (*S. cajanumense*) based on AFLP markers (according Dice coefficient). The *S. cajanumense* accession is indicated by an *asterisk*, while cultivar groups of *S. betaceum* by markers: orange (*open circles*), orange pointed (*solid circles*), purple (*solid square*), red (*solid triangle*). Accesions from Ecuador are underlined

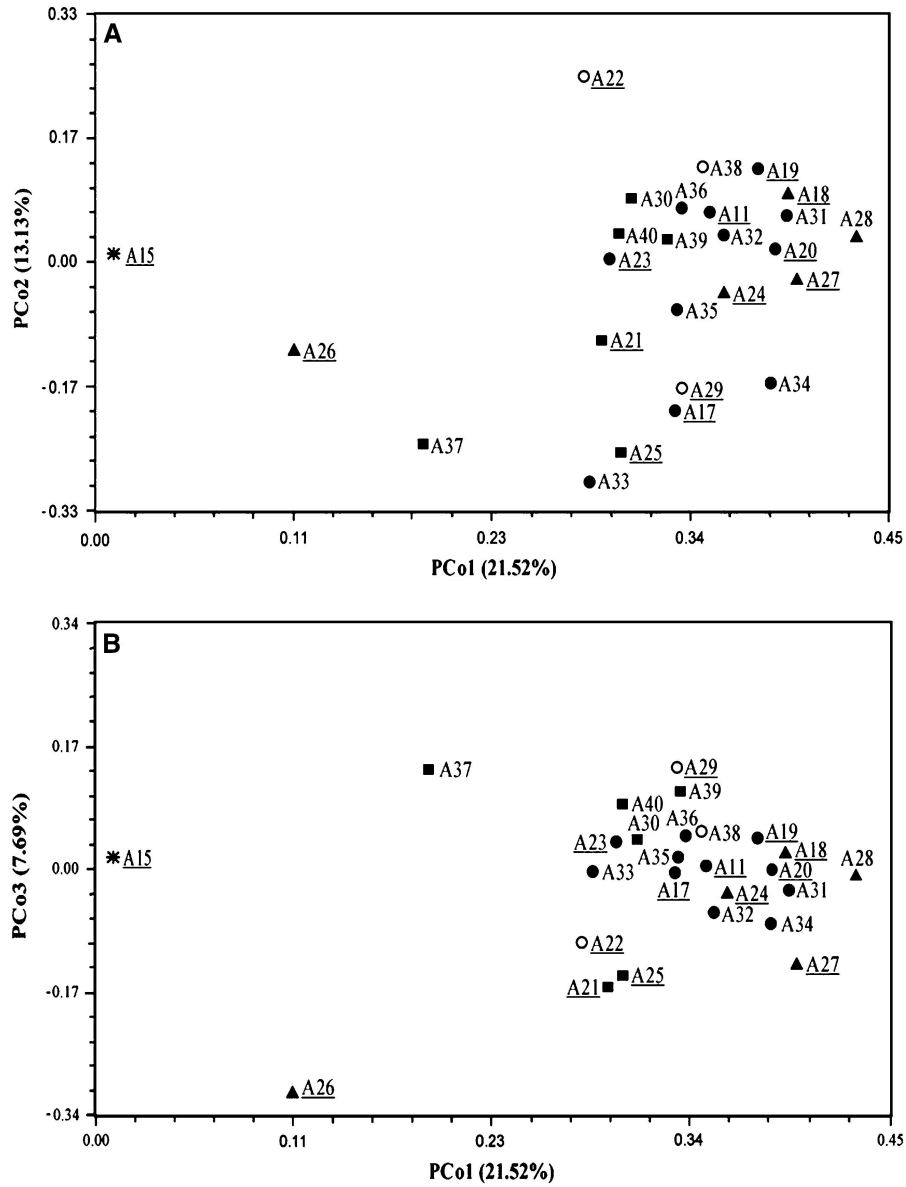

Fig. 2 Distribution of 25 cultivated tree tomato (*S. betaceum)* and one wild relative (*S. cajanumense*) accessions, according to the first (PCo1) and second (PCo2) principal coordinates (**a**), and on the first and third (PCo3) principal coordinates (**b**) of PCoA based on genetic similarities obtained from AFLP markers. PCo1, PCo2, and PCo3 account for 21.5, 13.1, and 7.7 %, respectively, of the total variation. The *S. cajanumense* accession is indicated by an *asterisk*, while cultivar groups of *S. betaceum* by markers: orange (*open circles*), orange pointed (*solid circles*), purple (*solid square*), red (*solid triangle*). Accesions from Ecuador are underlined

cultivar groups, H_T values ranged between 0.1846 (orange group) and 0.2498 (orange pointed group) (Table 4). Genetic differentiation (G_{ST}) among the four cultivar groups of *S. betaceum* was relatively low ($G_{ST} = 0.2248$), matched by the low values of genetic distance among cultivar groups (Table 5). The orange group presented the greatest values of genetic distance with other groups. When considering the genetic diversity according to geographical origin, we found that H_T of the material collected in Ecuador ($H_T = 0.2884$) was similar to that of the rest of origins combined ($H_T = 0.2645$) (Table 4). Genetic differentiation between accessions from Ecuador and those from other countries was very low ($G_{ST} = 0.0712$).

Multivariate analysis

The correlation coefficient between the cophenetic matrix of the UPGMA phenogram and that of genetic distances was 0.95, which is considered a high value (Rohlf 1996) and indicates a good agreement between

the phenogram and the genetic distances between pairs of accessions. The phenogram clearly separates the wild (*S. cajanumense*) A15 from the rest of accessions (Fig. 1). When considering only *S. betaceum* accessions, we found that the two basal branches separate accession A26 (red cultivar group, collected in Ecuador) from the rest. The branch containing the remaining 24 accessions is subdivided into two other sub-branches, one of which contains only two accessions (A39 and A40, both of the purple cultivar group and from New Zealand), whereas the other sub-branch, with 22 accessions, reveals that accessions of the different cultivar groups or origins are intermingled (Fig. 1). A phenogram excluding accession A15 showed a very similar topology.

The first three components of PCoA accounted for 21.5, 13.1, and 7.7 % of total variation (Fig. 2). The first coordinate separated accession A15 from the rest. In agreement with the UPGMA phenogram, the PCoA plot also shows that the first coordinate also separated accession A26 (red group, from Ecuador) from the rest of *S. betaceum* accessions (Fig. 2). This first coordinate also shows that accession A37 (purple group, from Portugal) is situated between A26 and the rest of accessions, which are clustered in the right part of the graph. Accessions included in this large group present a wide range of values of the second and third coordinates, but as occurred in the UPGMA phenogram, there is no evident separation of accessions by cultivar group or geographic origin (Fig. 2). Odd accessions A26 and A37, which present similar values of the second coordinate display very different values along the third coordinate (positive in A37 and negative in A26). In contrast to the UPGMA analysis, in which accessions A39 and A40 (purple group, New Zealand) constitute one of the basal sub-branches, these two accessions are here intermingled among accessions of the main group. A PCoA analysis excluding A15 (*S. cajanumense*) gives the same interpretation.

Comparison between morphological and molecular characterization

The availability of morphological characterization data from a former study (Acosta-Quezada et al. 2011) carried out with 22 *S. betaceum* accessions studied here allowed us to examine the correlation between the matrices of morphological and AFLP distances and AFLP similarities. The Mantel test correlation was of -0.024, a very low and non-significant ($P < 0.05$) value.

Discussion

Our work represents the first study of genetic diversity within and among different cultivar groups of tree tomato. It also allows comparing molecular data and morphological data based on criteria established by Acosta-Quezada et al. (2011). The accessions studied here represent a broad range of provenances along the Andean region, from which this crop originates (Bohs 1991; Bohs and Nelson 1997), as well as countries where the plant is also cultivated.

Given the lack of genomic information so far available for tree tomato, including a limited number (five) of polymorphic COS II markers (Enciso-Rodríguez et al. 2010) and a complete absence of SSRs and SNPs, the use of AFLP markers has proved appropriate for the study of genetic diversity in tree tomato. We found differences in the number of markers and degree of polymorphism among the combinations of primers used, which is typical with AFLPs (Meudt and Clarke 2007). For the eleven combinations of primers we scored a total of 78 polymorphic AFLP markers within *S. betaceum*, which has allowed us to study the diversity and relationships of cultivar groups and different origins. Similar studies in other *Solanum* crops, like potato or eggplant (*Solanum melongena* L.), examined the diversity and relationships of germplasm using less polymorphic AFLP markers (Kardolus et al. 1998; Veteläinen et al. 2005; Muñoz-Falcón et al. 2008a). However, it is also worth mentioning that the level of polymorphism found in cultivated tree tomato (38.6 %) has been lower than the value obtained in another neglected Andean *Solanum* species, pepino (*S. muricatum* Aiton), in which AFLP polymorphism reached 100 % (Blanca et al. 2007).

The five AFLP markers found to be monomorphic in *S. betaceum* were distinct from those found in *S. cajanumense*, which indicates that, as in other *Solanum* crops (Lara-Cabrera and Spooner 2004; Furini and Wunder 2004; Spooner et al. 2005; Blanca et al. 2007), AFLPs may be of great use for studying taxonomic relationships in section *Cyphomandropsis*. As in other crops, like eggplant (Muñoz-Falcón et al. 2008b, 2009), no specific and universal AFLP markers

have been found for cultivar groups of tree tomato. In this respect, SSRs have proved more useful to detect specific and universal markers for a cultivar group (García-Martínez et al. 2006; Muñoz-Falcón et al. 2008b, 2011). AFLPs produce a unique genetic profile for each of the studied accessions, which would be useful for the identification of cultivated and wild germplasm (Kardolus et al. 1998; Furini and Wunder 2004; Blanca et al. 2007). Furthermore, as in other *Solanum* crops (Rodríguez-Burruezo et al. 2003, 2008), the use of AFLP based genetic distances among accessions could be useful for obtaining heterotic hybrids of tree tomato, by selecting parents situated at large genetic distances.

The genetic diversity found in tree tomato shows that there is considerable diversity within cultivar groups, and a relatively low genetic differentiation among groups, which has important implications for the conservation of genetic resources and breeding of tree tomato (Soller and Beckmann 1983). For example, these results indicate that an important part of the diversity in the species could be preserved by conserving only a few accessions, even if they belong to the same cultivar group.

When Ecuadorian versus other origins are compared, similar levels of genetic diversity and a low level of genetic differentiation are found, showing that despite the fact that Bolivia is considered the center of origin of this crop (Bohs 1991; Bohs and Nelson 1997), Ecuador contains a high level of diversity, indicating that this country is a center of accumulation of diversity (Harlan 1992) for tree tomato. The low genetic differentiation among geographical origins may be a consequence of prolonged exchange and trade of seeds and fruits among different regions. Also, the great range of different environments and microclimates along the Andean region, even within the same geographical area, would have favored the accumulation of diversity and lack of genetic differentiation among regions.

Both UPGMA and PCoA analyses show that the *S. cajanumense* accession is clearly differentiated from *S. betaceum*. However, they show that different cultivar groups, or accessions from different geographical origins, do not cluster in separate branches in the UPGMA phenogram or areas in the PCoA plot. The lack of clear grouping of accessions according to cultivar groups or origins together with a low genetic differentiation are commonly found in other *Solanum* crops, like potato (Veteläinen et al. 2005), tomato (Mazzucato et al. 2008), eggplant (Prohens et al. 2005), or pepino (Blanca et al. 2007). The results obtained from UPGMA and PCoA analyses are largely in agreement. The small differences are only the result of the different methodologies employed in both types of multivariate analyses and point out the complementarity of both approaches to study relationships among accessions (Mohammadi and Prasanna 2003).

Correlations between morphological and molecular data are usually very variable and dependent on the crop, plant material, morphological descriptors and molecular markers used (Mohammadi and Prasanna 2003). The correlation between the morphological distances obtained from 39 quantitative morphological descriptors (Acosta-Quezada et al. 2011) and the molecular distances resulting from 78 polymorphic AFLP markers of 22 accessions of cultivated tree tomato in our study is not significant. Lack of morphological-molecular correlation has been found in other solanaceous crops, like potato (Veteläinen et al. 2005) or pepper (Geleta et al. 2005), as well as in others, like olive tree (*Olea europaea* L.) (Hagidimitriou et al. 2005), barley (*Hordeum vulgare* L.) (Lund 2002), or sorghum (*Sorghum bicolor* (L.) Moench) (Geleta et al. 2006). The lack of correlation between the two data sets suggests that morphological descriptors and AFLP data sample different levels of diversity in this crop and in consequence the information obtained from both types of data is complementary and in any case useful for the conservation of germplasm and breeding of tree tomato. Morphological markers sample traits for which variation may be controlled by few genes (Doganlar et al. 2002), while AFLPs mostly detect point mutations in non-codifying regions of the genome (Meudt and Clarke 2007). Wendel and Doyle (1998) suggested that the lack of correlation between morphological and genetic distances may be caused by the fact that morphological and molecular evolutions follow different paths. In consequence, the use of both types of data in tree tomato is convenient for managing germplasm collections and for the establishment of nuclear collections (Brown 1995; Ghislain et al. 1999). Also, it has been demonstrated in other solanaceous crops, like eggplant (Rodríguez-Burruezo et al. 2008), that AFLP genetic distances are better than morphological distances to select parents to obtain heterotic hybrids. It remains to be investigated if this is also the case in tree tomato.

In conclusion, AFLPs are useful for tree tomato germplasm fingerprinting and reveal that a wide diversity is present in each of the cultivar groups, which show a low level of genetic differentiation. The high diversity found in Ecuadorian accessions indicates that this country can be considered a center of accumulation of diversity for this crop. Finally, the lack of correlation between morphological and AFLP data shows that in tree tomato both types of data are complementary. The results obtained are of interest for the conservation of genetic resources and breeding of this promising neglected Andean crop.

Acknowledgments This work was partially financed by the Ministerio de Ciencia e Innovación (RF2008-00008-00-00) as well as by the Secretaría Nacional de Educación Superior, Ciencia, Tecnología e Innovación from Ecuador (SENESCYT).

References

Acosta-Quezada PG, Martínez-Laborde JB, Prohens J (2011) Variation among tree tomato (*Solanum betaceum* Cav.) accessions from different cultivar groups: implications for conservation of genetic resources and breeding. Genet Resour Crop Evol 58:943–960

Benham J, Jeung JU, Jasieniuk M, Kanazin V, Blake T (1999) Genographer: a graphical tool for automated fluorescent AFLP and microsatellite analysis. Department of Plant Science, Montana State University, Bozeman

Blanca J, Prohens J, Anderson GJ, Zuriaga E, Cañizares J, Nuez F (2007) AFLP and DNA sequence variation in an Andean domesticate pepino (*Solanum muricatum, Solanaceae*): implications for evolution and domestication. Am J Bot 94:1219–1229

Bohs L (1989) Ethnobotany of the genus *Cyphomandra* (Solanaceae). Econ Bot 43:143–163

Bohs L (1991) Crossing studies in *Cyphomandra* (Solanaceae) and their systematic and evolutionary significance. Am J Bot 78:1683–1693

Bohs L, Nelson A (1997) *Solanum maternum* (Solanaceae), a new Bolivian relative of the tree tomato. Novon 7:341–345

Boyes S, Strübi P (1997) Organic acid and sugar composition of three New Zealand grown tamarillo varieties (*Solanum betaceum* (Cav.)). N Z J Crop Hortic Sci 25:79–83

Brown AHD (1995) The core collection at the crossroads. In: Hodgkin T, Brown AHD, van Hintum TJL, Morales EAV (eds) Core collections of plant genetic resources. John Wiley & Sons, Chichester, pp 3–19

Cohen D, van den Brink RC, MacDiarmid RM, Beck DL, Forster RLS (2000) Resistance to *Tamarillo mosaic virus* in transgenic tamarillos and expression of the transgenes in F1 progeny. Acta Hortic 521:43–49

Doganlar S, Frary A, Daunay MC, Lester RN, Tanksley SD (2002) Conservation of gene function in the Solanaceae as revealed by comparative mapping of domestication traits in eggplant. Genetics 161:1713–1726

Doyle JJ, Doyle JL (1987) A rapid isolation procedure for small quantities of fresh leaf tissue. Phytochem Bull 19:11–15

Enciso-Rodríguez F, Martínez R, Lobo M, Barrero LS (2010) Genetic variation in the Solanaceae fruit bearing species lulo and tree tomato revealed by Conserved Ortholog (COSII) markers. Genet Mol Biol 33:271–278

Furini A, Wunder J (2004) Analysis of eggplant (*Solanum melongena*)-related germplasm: morphological and AFLP data contribute to phylogenetic interpretations and germplasm utilization. Theor Appl Genet 108:197–208

García-Martínez S, Andreani L, García-Gusano M, Geuna F, Ruiz JJ (2006) Evaluation of amplified fragment length polymorphism and simple sequence repeats for tomato germplasm fingerprinting: utility for grouping closely related traditional cultivars. Genome 49:648–656

Geleta LF, Labuschagne MT, Viljoen CD (2005) Genetic variability in pepper (*Capsicum annuum* L.) estimated by morphological data and amplified fragment length polymorphism markers. Biodivers Conserv 14:2361–2375

Geleta N, Labuschagne MT, Viljoen CD (2006) Genetic diversity analysis in sorghum germplasm as estimated by AFLP, SSR and morpho-agronomical markers. Biodivers Conserv 15:3251–3265

Ghislain M, Zhang D, Fajardo D, Huamán Z, Hijmans RJ (1999) Marker-assisted sampling of the cultivated Andean potato *Solanum phureja* collection using RAPD markers. Genet Resour Crop Evol 46:547–555

Hagidimitriou M, Katsiotis A, Menexes G, Pontikis C, Loukas M (2005) Genetic diversity of major Greek olive cultivars using molecular (AFLPs and RAPDs) markers and morphological traits. J Am Soc Hortic Sci 130:211–217

Harlan JR (1992) Crops and man. American Society of Agronomy and Crop Science Society of America, Madison

Hillis DM (1987) Molecular versus morphological approaches to systematics. Annu Rev Ecol Syst 18:23–42

Jones CJ, Edwards KJ, Castiglione S, Winfield WO, Sala F, van de Wiel C, Bredemeijer G, Vosman B, Matthes M, Daly A, Brettschneider R, Bettini P, Buiatti M, Maestri B, Malcevschi A, Marmiroli N, Aert R, Volckaert G, Rueda J, Linacero R, Vázquez A, Karp A (1997) Reproducibility testing of RAPD, AFLP and SSR markers in plants by a network of European laboratories. Mol Breed 3:381–390

Kardolus JP, van Eck HJ, van den Berg RG (1998) The potential of AFLPs in biosystematics: a first application in *Solanum* taxonomy (Solanaceae). Plant Syst Evol 210:87–103

Lara-Cabrera SI, Spooner DM (2004) Taxonomy of North and Central American diploid wild potato (*Solanum* sect. *Petota*) species: AFLP data. Plant Syst Evol 248:129–142

Lund B (2002) Repatriation of Nordic barley germplasm. PhD dissertation, The Royal Veterinary and Agricultural University, Section of Plant Breeding and Crop Science, Copenhagen, Denmark

Mantel N (1967) The detection of disease clustering and generalized regression approach. Cancer Res 27:209–220

Mazzucato A, Papa R, Bitocchi E, Mosconi P, Nanni L, Negri V, Picarella ME, Siligato F, Soressi GP, Tiranti B, Veronesi F (2008) Genetic diversity, structure and marker-trait associations in a collection of Italian tomato (*Solanum lycopersicum* L.) landraces. Theor Appl Genet 116:657–669

Meudt HM, Clarke AC (2007) Almost forgotten or latest practice? AFLP applications, analyses and advances. Trends Plant Sci 12:106–117

Milbourne D, Meyer R, Bradshaw JE, Baird E, Bonar N, Provan J, Powell W, Waugh R (1997) Comparison of PCR-based marker systems for the analysis of genetic relationships in cultivated potato. Mol Breed 3:127–136

Mohammadi SA, Prasanna BM (2003) Analysis of genetic diversity in crop plants—salient statistical tools and considerations. Crop Sci 43:1235–1248

Muñoz-Falcón J, Prohens J, Vilanova S, Nuez F (2008a) Characterization, diversity, and relationships of the Spanish striped (*Listada*) eggplants: a model for the enhancement and protection of local heirlooms. Euphytica 164:405–419

Muñoz-Falcón J, Prohens J, Vilanova S, Ribas F, Castro A, Nuez F (2008b) Distinguishing a protected geographical indication vegetable (*Almagro* eggplant) from closely related varieties with selected morphological traits and molecular markers. J Sci Food Agric 89:320–328

Muñoz-Falcón J, Prohens J, Vilanova S, Nuez F (2009) Diversity in commercial varieties and landraces of black eggplants and implications for broadening the breeders' gene pool. Ann Appl Biol 154:453–465

Muñoz-Falcón J, Vilanova S, Plazas M, Prohens J (2011) Diversity, relationships and genetic fingerprinting of the *Listada de Gandía* eggplant landrace using genomic SSRs and EST-SSRs. Sci Hortic 129:238–246

National Research Council (1989) Lost crops of the Incas: little-known plants of the Andes with promise for world-wide cultivation. National Academy Press, Washington, DC

Nei M (1972) Genetic distance between populations. Am Nat 106:283–292

Nei M (1973) Analysis of gene diversity in subdivided populations. Proc Natl Acad Sci USA 70:3321–3323

Portis E, Baudino M, Magurno F, Lanteri S (2012) Genetic structure and preservation strategies of autochtonous vegetable crop landraces of north-western Italy. Ann Appl Biol 160:76–85

Powell W, Morgante M, Andre C, Hanafey M, Vogel J, Tingey S, Rafalski A (1996) The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. Mol Breed 2:225–238

Pringle GJ, Murray BG (1991) Interspecific hybridization involving the tamarillo, *Cyphomandra betacea* (Cav.) Sendt. (Solanaceae). N Z J Crop Hortic Sci 19:103–111

Prohens J, Nuez F (2000) The tamarillo (*Cyphomandra betacea*): a review of a promising small crop. Small Fruits Rev 1(2):43–68

Prohens J, Rodríguez-Burruezo A, Nuez F (2004) Breeding Andean Solanaceae fruit crops for adaptation to subtropical climates. Acta Hortic 662:129–137

Prohens J, Blanca J, Nuez F (2005) Morphological and molecular variation in a collection of eggplants from a secondary center of diversity: implications for conservation and breeding. J Am Soc Hortic Sci 130:54–63

Rodríguez-Burruezo A, Prohens J, Nuez F (2003) Performance of hybrid segregating populations of pepino (*Solanum muricatum*) and its relation to genetic distance among parents. J Hortic Sci Biotechnol 78:911–918

Rodríguez-Burruezo A, Prohens J, Nuez F (2008) Performance of hybrids between local varieties of eggplant (*Solanum melongena*) and its relation to the mean of parents and to morphological and genetic distances among parents. Eur J Hortic Sci 73:76–83

Rohlf FJ (1996) NTSYS-pc: numerical taxonomy and multivariate system, version 2.0. Exeter Software, Setauket

Sneath PHA, Sokal RR (1973) Numerical taxonomy. W. H. Freeman, San Francisco

Soller M, Beckmann J (1983) Genetic polymorphism in varietal identification and genetic improvement. Theor Appl Genet 67:25–33

Spooner DM, Peralta IE, Knapp S (2005) Comparison of AFLPs with other markers for phylogenetic inference in wild tomatoes [*Solanum* L. section *Lycopersicon* (Mill.) Wettst.]. Taxon 54:43–61

Tam SM, Mhiri C, Vogelaar A, Kerkveld M, Pearce SR, Grandbastien MA (2005) Comparative analyses of genetic diversities within tomato and pepper collections detected by retrotransposon-based SSAP, AFLP and SSR. Theor Appl Genet 110:819–831

Vasco C, Avila J, Ruales J, Svanberg U, Kamal-Eldin A (2009) Physical and chemical characteristics of golden-yellow and purple-red varieties of tamarillo fruit (*Solanum betaceum* Cav.). Int J Food Sci Nutr 60:278–288

Veteläinen M, Gammelgard E, Valkonen JPT (2005) Diversity of Nordic landrace potatoes (*Solanum tuberosum* L.) revealed by AFLPs and morphological characters. Genet Resour Crop Evol 52:999–1010

Wendel JF, Doyle JJ (1998) Phylogenetic incongruence: window into genome history and molecular evolution. In: Soltis D, Soltis P, Doyle J (eds) Molecular systematics of plants II: DNA sequencing. Kluwer Academic Publishers, Norwell, pp 265–296

Yeh FC, Boyle TJB (1997) Population genetic analysis of codominant and dominant markers and quantitative traits. Belgian J Bot 129:157